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Distribution and Bioaccumulation of Selenium in Aquatic Microcosms

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ABSTRACT

Closed-system microcosms were used to study factors affecting the fate of selenium (Se) in aquatic systems. Distribution and bioaccumulation of Se varied among sediment types and Se species. A mixture of dissolved ^{75}Se species (selenate, selenite and selenomethionine) was sorbed more rapidly to fine-textured, highly organic pond sediments than to sandy riverine sediments. Sulfate did not affect the distribution and bioaccumulation of ^{75}Se over the range 80–180 mg SO_4 liter^{-1}. When each Se species was labeled separately, selenomethionine was lost from the water column more rapidly than selenate or selenite. Selenium lost from the water column accumulated primarily in sediments, but volatilization was also an important pathway for loss of Se added as selenomethionine. Loss rates of dissolved Se residues were more rapid than rates reported from mesocosm and field studies, suggesting that sediment: water interactions are more important in microcosms than in larger test systems. Daphnids accumulated highest concentrations of Se, followed by periphyton and macrophytes. Selenium added as selenomethionine was bioaccumulated preferentially compared to that added as selenite or selenate. Organoselenium compounds such as selenomethione may thus contribute disproportionately to Se bioaccumulation and toxicity in aquatic organisms.

INTRODUCTION

Selenium contamination of lakes and reservoirs from agricultural drainage and fossil fuel combustion has been linked to toxic effects in fish and

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waterfowl (Gillespie & Baumann, 1986; Ohlendorf *et al.*, 1986). The fate and bioavailability of Se in aquatic ecosystems may be affected by a variety of physico-chemical and biotic factors: characteristics of suspended solids and sediments affect sorption rates of dissolved Se (Hesslein *et al.*, 1980); interactions with dissolved constituents such as sulfate affect Se bioavailability (Schrift, 1973; Rudd *et al.*, 1980); and aquatic organisms accumulate high body burdens of Se and produce organoselenium compounds that may be more toxic than inorganic forms (Sandholm *et al.*, 1973, Cooke & Bruland, 1987).

Our study of the fate and bioaccumulation of Se in laboratory microcosms had two objectives: to evaluate the influence of sediment characteristics, sulfate concentrations, and Se speciation on the distribution and bioaccumulation of Se in microcosms; and to compare the results of microcosm studies with results of field studies of Se-contaminated aquatic systems.

MATERIALS AND METHODS

Experiments were conducted in closed-system microcosms modified from Huckins *et al.* (1984). Microcosm studies were conducted in an environmental chamber at 24°C and controlled photoperiod (16 h light:8 dark). Microcosms consisted of pyrex containers equipped with Teflon head assemblies that allowed the removal of water samples and insertion of probes for water quality analysis. Each microcosm received wet sediment (50 g dry weight) and water to a total volume of 1 liter, leaving about 100 ml of headspace above the water surface. Airflow through the headspace passed through columns containing activated carbon (Calgon PCB carbon, Calgon Corp., Hinsdale, Illinois; and Spherocarb©, Analabs, Norwalk, Connecticut) to trap volatile organic compounds. Low air flow rates (approx. 100 ml min^{-1}) were maintained to prevent pressure buildup from influencing volatilization rates. One week after the addition of sediment and water, the microcosms were stocked with 10 juvenile *Daphnia magna* and 10^8 cells of *Selenastrum capricornutum*; after 24 h they were spiked with low doses of Se compounds in known ratios of ^{75}Se-radiolabeled (Amersham Inc., Arlington Heights, Illinois) and non-radioactive Se.

Microcosms were randomly assigned to treatment groups in two studies. The first study compared the influences of sediment characteristics and dissolved sulfate concentrations on the distribution and bioaccumulation of a simulated natural mixture of Se compounds. Each of 12 microcosms received one of two sediment types and one of two sulfate concentrations (3 microcosms for each combination of sediment type and sulfate con-

centration). Sources of the two sediment types were a mid-channel site in San Joaquin River near Los Banos, California ('San Joaquin' sediment), and a pond on the Volta Wildlife Area, California ('Volta' sediment). Sediments were shipped on ice and refrigerated until needed; storage time was 3–5 months. Volta sediment had a higher proportion of silt- and clay-sized particles, higher organic content, and higher nutrient levels than San Joaquin sediment, but a glucose mineralization assay (modified from Johnson, 1986) indicated similar levels of microbial activity in the two sediments (Table 1). Atomic absorption analysis (May, 1982) found low but detectable Se residues in the Volta sediment (Table 1). Dissolved Se concentrations in control microcosms, which received sediments but no added Se, were below detection ($\leq 1\,\mu g$ Se liter^{-1}) indicating little or no release of Se from either sediment. Reconstituted test water with ionic composition similar to that of water in the San Joaquin River (pH 8·04, sulfate 74 mg liter^{-1}, alkalinity and hardness 70 and 136 mg liter^{-1} as $CaCO_3$, respectively) was added to microcosms without added sulfate ('low sulfate' treatment) or with 100 mg liter^{-1} added sulfate ('high sulfate' treatment). Water volumes added to produce 1 liter total volume averaged 954 ml for microcosms with San Joaquin sediment and 921 ml for microcosms with Volta sediment, due to the greater wet volume of the Volta sediment. Microcosms in the first study received a mixture of three selenium species based on their proportions in contaminated drainwaters in the San Joaquin Valley: sodium selenate (Se^{+6}), 34 μg Se liter^{-1}; sodium selenite (Se^{+4}), 5·7 μg Se liter^{-1}; and seleno-L-methionine (Se^{-2}), 1·9 μg Se liter^{-1}. An equal activity of each ^{75}Se-labeled species (2 μCi each; 6 μCi per microcosm) was added to each microcosm to allow detection of trends in the fate and bioconcentration of each species. This approach resulted in a disproportionate emphasis on the contributions of selenomethionine and

TABLE 1
Characteristics of Sediments used in Microcosm Studies[a]

Collection site (study)	*Organic carbon (%)*	*Silt + clay (%)*	*Glucose mineralization (cpm g day^{-1})*	*Total N (mg kg^{-1})*	*Total P (mg kg^{-1})*	*Total Se (mg kg^{-1})*
San Joaquin (Study 1)	0·05	36·2	3 906	156	184	≤0·04
Volta (Study 1)	2·16	87·3	3 699	2 450	332	0·49
San Joaquin (Study 2)	0·35	26·3	12 173[b] 20 043[c]	318	519	≤0·04

[a] Pre-study means on dry wt basis, $N = 2$ unless noted otherwise.
[b] $N = 6$.
[c] Post-study measurement, $N = 12$.

selenite relative to selenate, due to different ratios of radioactive to nonradioactive isotopes for each species (Se^{-2}, 1·05 μCi/μg; Se^{+4}, 0·35 μCi/μg; Se^{+6}, 0·06 μCi/μg.

The second study was designed to compare differences in the fate and bioconcentration of the three Se species. Nine microcosms were divided into groups of three and each group received only one of the three Se species as ^{75}Se (3 μCi of ^{75}Se per microcosm). Each microcosm received all three nonlabeled species to produce the same total Se concentrations and proportions of Se species used in the first study. All microcosms in the second study received reconstituted water (without added sulfate) and sediment from a depositional site in the San Joaquin River. The sediment used in this study was similar to the San Joaquin sediment used in the first study, except for slightly greater organic carbon and nutrient content, and greater heterotrophic activity (Table 1).

Samples of microcosm components and biota were collected to measure Se fate and bioaccumulation during and after 28-day studies. Water, suspended solids and volatiles were sampled during the course of the study (13 water samples and 4 weekly composite samples of volatiles). Replicate subsamples of sediment and complete samples of biota were collected at the end of the studies. The biota sampled included zooplankton (stocked daphnids and their offspring); periphyton (algae and associated material attached to the wall of the microcosm); and macrophytes (rooted plants and filamentous algae). No attempt was made to differentiate these samples taxonomically. We measured ^{75}Se activity on a Beckman Model 8000 gamma counter, adjusted counts for radioactive decay from the beginning of the study, and calculated ^{75}Se activity and Se concentrations on a dry weight (sediment, biota) or volume (water) basis. Microcosm volumes used in recovery calculations were adjusted for sample removal. Recovery of added ^{75}Se averaged 87% in the first study. Recovery of selenomethionine averaged 89% in the second study, compared to 100% for selenate and selenite, suggesting that incomplete recovery in both studies was due to escape of volatile metabolic products of selenomethionine. Percentage distribution of ^{75}Se among water, sediment, air and biota was estimated by comparison with total recovery from each microcosm. Bioconcentration factors (BCFs), ratios of organism Se concentration to dissolved Se concentration, were calculated using average dissolved Se concentrations during the final 14 days of the second study to approximate equilibrium dissolved Se concentrations. Dissolved oxygen and pH were monitored with an Orion Model 901 Ionalyzer system; primary production was estimated by the diurnal O_2 curve method (McConnell, 1962); and sulfate concentrations were measured using an automated colorimetric method (American Public Health Association 1976) (Table 2).

TABLE 2
Water Quality During Microcosm Studies[a]

Study 1

Variable	*N*	*San Joaquin*	*Volta*	*High-SO_4*	*Low-SO_4*
pH	54	8·45	9·09	8·78	8·76
DO_{max}[b]	12	10·10	11·61	10·85	10·86
DO_{min}[c]	42	9·60	10·14	10·06	9·68
GPP[d]	42	3·11	5·56	4·04	4·63
SO_4 (mg liter^{-1})	42	124	143	185	82

Study 2

Variable	*N*	*Selenomethionine*	*Selenite*	*Selenate*
pH	18	8·70	8·57	8·81
DO_{max}	18	10·34	9·78	10·21
DO_{min}	18	9·18	8·79	9·11
GPP	18	3·57	3·49	4·22

[a] Means by treatment group.
[b,c] Dissolved oxygen daily maximum (*b*) and minimum (*c*), mg liter^{-1}.
[d] Gross primary production, mg O_2 liter^{-1} day^{-1}.

Comparisons of Se distribution and bioaccumulation among treatments were made by analysis of variance (ANOVA); data were rank-transformed to equalize variances among treatments (Conover & Iman, 1981). Two-way rank ANOVA was used to determine effects of sediment and sulfate treatments in the first study; differences among Se compounds in the second study were tested with one-way rank ANOVA (Kruskal-Wallis test) with multiple comparisons of rank means made with Fisher's LSD procedure. Changes in dissolved Se concentrations over time were modeled with linear or polynomial regression (Neter & Wasserman, 1974). Statements of statistical significance refer to a 5% Type I error rate ($p \leq 0{\cdot}05$).

RESULTS

Dissolved Se concentrations in microcosms were affected by sediment type and Se speciation. Decreases in dissolved ^{75}Se concentrations were initially rapid, then became more gradual (Fig. 1A). After the first 3 days, dissolved ^{75}Se decreased more rapidly in microcosms with Volta sediments than in microcosms with San Joaquin sediments. Concentrations of dissolved ^{75}Se

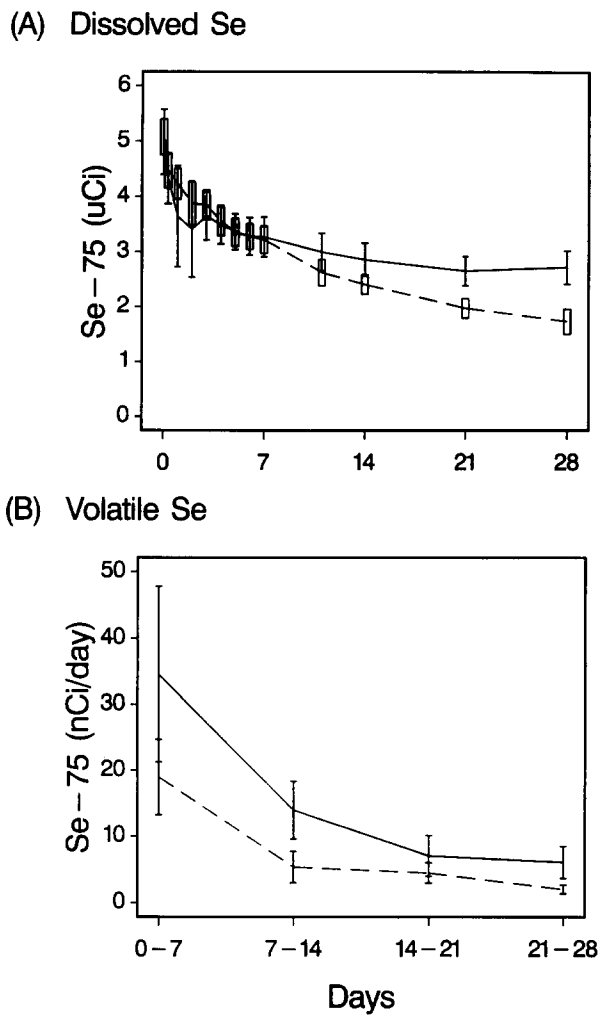

Fig. 1. Dissolved and volatile Se in microcosms during Study 1. Mean ^{75}Se activities ± standard error. Solid line = San Joaquin sediment; dashed line = Volta sediment. $N = 5$ microcosms per sediment type. A. Dissolved Se (μCi per microcosm). B. Volatile Se (nCi per microcosm day^{-1}).

were significantly higher in the San Joaquin sediment treatment during the final 14 days of the study. The fine-textured, highly organic Volta sediment accumulated significantly more ^{75}Se than did the San Joaquin sediment (Table 3). Dissolved ^{75}Se activity did not differ significantly between the high- and low-sulfate treatments, but sediment accumulated significantly more ^{75}Se in the low-sulfate treatment. Differences in sediment ^{75}Se accumulation between sulfate treatments (3%) were much less than differences between sediment treatments (30%).

TABLE 3
Distribution and Bioaccumulation of ^{75}Se after 28 Days in Study 1[a]

Variable and sample type	*San Joaquin sediment*		*Volta sediment*	
	Low-SO_4 ($N = 2$)	*High-SO_4* ($N = 3$)	*Low-SO_4* ($N = 3$)	*High-SO_4* ($N = 2$)
Se Distribution (%)				
Volatile[b]	7·9 ± 1·8	7·2 ± 1·2	4·6 ± 0·3	3·0 ± 0·5
Dissolved[b]	48·9 ± 1·7	54·5 ± 2·0	29·6 ± 0·2	28·2 ± 1·7
Suspended[b]	3·4 ± 0·3	3·3 ± 0·2	1·7 ± 0·04	1·7 ± 0·1
Sediment[b,c]	35·3 ± 2·3	31·4 ± 0·8	62·4 ± 0·2	61·1 ± 1·9
Biota	4·5 ± 1·8	3·6 ± 1·2	1·6 ± 0·2	5·9 ± 0·5
Se Bioaccumulation ($\mu Ci\,g^{-1}$)				
Macrophytes[f]	0·06	0·05 ± 0·03	0·39 ± 0·02	0·22 ± 0·13
Periphyton	2·49 ± 0·12	1·10 ± 0·19	0·74 ± 0·08	1·26 ± 0·08
Zooplankton	5·04[d]	2·76 ± 0·52	1·20 ± 0·78[e]	2·95 ± 0·20

[a] Means by treatment group ± standard error.
[b,c] Significant difference between (*b*) sediment types and/or (*c*) sulfate levels determined by 2-way rank ANOVA.
[d] $N = 1$ (sample recovered from only 1 microcosm).
[e] $N = 2$.

Dissolved selenomethionine residues decreased more rapidly than residues of selenate and selenite (Fig. 2A). Concentrations of ^{75}Se in the selenomethionine treatment decreased rapidly early in the study, then leveled off, but remained significantly lower than in the selenate or selenite treatments throughout the study. Concentrations of ^{75}Se in the selenite treatment were significantly lower than in the selenate treatment. The decreasing portion of the selenomethionine loss curve (days 1–11) and the entire curves for selenate and selenite were approximated by exponential decrease models. These regressions differed significantly among the three Se species: half-times of dissolved ^{75}Se residues were 7 days for selenomethionine, 20 days for selenite, and 33 days for selenate. Differences in loss rates of dissolved ^{75}Se between selenate and selenite treatments were apparently due to greater sediment accumulation of selenite residues, but sediment Se accumulation was similar in the selenite and selenomethionine treatments (Table 4).

Volatilization rates of Se residues also differed among sediment types and Se species. Volatilization rates of the mixture of Se species were initially high, but declined rapidly in microcosms with both sediment types (Fig. 1B). The proportion of added ^{75}Se lost to volatilization was significantly greater in the San Joaquin sediment treatment (Table 3). Volatilization did not differ

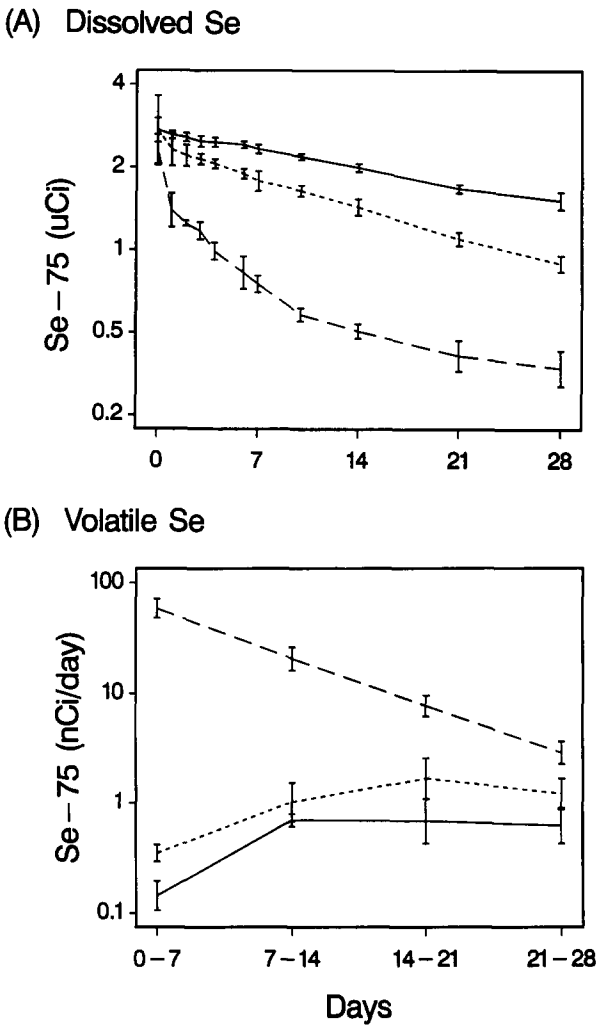

Fig. 2. Dissolved and volatile Se in microcosms during Study 2. Mean ^{75}Se activities ± standard error, plotted on logarithmic scale. Solid line = selenate, dotted line = selenite, dashed line = Se-methionine. $N = 3$ microcosms per Se species. A. Dissolved Se (μCi/microcosm). B. Volatile Se (nCi per microcosm day^{-1}).

between low- and high-sulfate treatments. Selenium volatilization was initially rapid in the selenomethionine treatment but decreased throughout the study, whereas volatilization increased gradually from very low initial rates in the selenite and selenate treatments (Fig. 2B). The volatilization rate remained significantly higher in the selenomethionine treatment throughout the study. Overall, volatilization accounted for 24% of ^{75}Se added as selenomethionine, compared to 1% or less of that added as selenate and

TABLE 4

Distribution and Bioaccumulation of ^{75}Se after 28 Days in Study 2. Bioconcentration Factor = Se Concentration in Organisms Divided by Se Concentration in Water[a]

Variable and sample type	*Selenate*	*Selenite*	*Se-methionine*
Se distribution (%)			
Volatiles[b]	0·5 ± 0·03	1·1 ± 0·2	24·0 ± 1·7
Dissolved[b]	50·0 ± 1·6	32·0 ± 2·5	13·9 ± 1·0
Suspended	2·2 ± 0·1	2·9 ± 0·4	3·2 ± 0·5
Sediment[b]	46·8 ± 1·5	62·7 ± 2·7	55·7 ± 1·1
Biota[b]	0·5 ± 0·1	1·2 ± 0·4	3·2 ± 0·5
Se concentration			
Water (μg liter^{-1})	19·0 ± 0·4	2·5 ± 0·3	0·3 ± 0·01
Macrophytes ($\mu g\, g^{-1}$)	1·3 ± 0·6	1·0 ± 0·6[c]	0·9 ± 0·2
Periphyton ($\mu g\, g^{-1}$)	2·7 ± 0·4	1·8 ± 0·3	4·3 ± 1·7
Zooplankton ($\mu g\, g^{-1}$)	6·7 ± 0·9	2·5 ± 1·3	7·5 ± 2·2
Bioconcentration factor			
Macrophytes[b]	72 ± 32	363 + 147[c]	3 266 ± 535
Periphyton[b]	141 ± 22	755 ± 138	16 836 ± 6 738
Zooplankton[b]	351 ± 42	1 087 ± 611	28 870 ± 9 369

[a] Means ± standard error; $N = 3$ unless otherwise indicated.
[b] Significant difference among treatments (rank ANOVA) and in all pairwise mean comparisons (Fisher's LSD).
[c] $N = 2$.

selenite (Table 4). This suggests that differences in rates of Se volatilization among sediment treatments were due to differences in volatilization of the selenomethionine portion of the Se mixture.

Selenium bioaccumulation differed among biotic components and among treatment groups. Bioaccumulation of ^{75}Se was greater in daphnids than in periphyton or macrophytes (Tables 3 and 4). Bioaccumulation of ^{75}Se by daphnids and periphyton did not differ between sediment or sulfate treatments, but macrophytes accumulated significantly higher ^{75}Se concentrations in microcosms with Volta sediment, perhaps due to greater ^{75}Se accumulation in this sediment (Table 3). Selenium was accumulated much more readily by biota from selenomethionine than from selenite or selenate (Table 4). Bioconcentration factors (BCFs) were significantly different among the three Se species. Although this difference may be due in part to higher BCFs at low dissolved Se concentrations, zooplankton and periphyton actually accumulated greater Se concentrations from selenomethionine than from selenite or selenate, despite higher dissolved concentrations of the inorganic Se species.

DISCUSSION

Selenium fate in closed-system microcosms was strongly affected by differences in sediment sorption and bioaccumulation of Se species. Our results suggest that sediment characteristics influence the fate of dissolved Se in shallow aquatic systems with high sediment surface area/volume ratios. This effect was not observed in high-volume *in situ* lake enclosures (Rudd *et al.*, 1980). The fine texture and high organic content of the Volta sediment apparently favored Se sorption relative to the coarse, inorganic San Joaquin sediment. Selenium uptake by sediment-dwelling organisms and sedimentation of Se-enriched detritus, both favored by higher nutrient levels, may also have contributed to Se accumulation in the Volta sediment. The presence of Se-adapted microflora could have increased Se accumulation in sediment, but selenite-resistant strains are rare in Volta sediments (Burton *et al.*, 1987). Although increasing sulfate concentrations from 10 to 78 mg liter^{-1} resulted in increased Se bioaccumulation in lake enclosures (Rudd *et al.*, 1980), an increase in sulfate between 80 and 180 mg liter^{-1} did not affect selenium fate or bioaccumulation in our microcosms. Decreased Se uptake would be expected at high sulfate concentrations due to sulfate:selenate antagonism (Shrift, 1973), but this effect would have been masked by the low proportion of selenate to total ^{75}Se activity in our microcosms.

Selenate and selenite were more persistent than selenomethionine in the water column of microcosms, but were less readily accumulated by aquatic organisms. Loss rates of dissolved Se species from water increased in the order: selenate < selenite < selenomethionine. The loss rate of Se added as selenite was about twice that reported from a selenite addition to an entire lake (Hesslein *et al.*, 1980), but followed a similar exponential decrease curve. Differences in the loss rates of the three Se species from water were apparently caused by differences in sediment sorption (greater for selenite and selenomethionine) and bioaccumulation and volatilization (greater for selenomethionine). However, differences in the total amounts of each Se species added to our microcosms ($Se^{+6} > Se^{+4} > Se^{-2}$) may also have contributed to differences in Se loss rates.

Bioconcentration factors and volatilization rates were roughly proportional to loss rates from water. Selenium volatilization, primarily of alkylated selenides, has been linked to Se biotransformation and release by aquatic organisms (Cooke & Bruland, 1987). Unpublished data from our laboratory support this assertion, as no volatilization of selenate, selenite, or selenomethionine occurred in sterile microcosms. The lower BCFs and volatilization rates of Se added as selenate and selenite suggest that bioaccumulation and biotransformation of these species are slower than that of selenomethionine. Selenium BCFs in microcosms spiked with

selenite were similar to those reported for selenite in other model ecosystems (Nassos *et al.*, 1980), but were greater than those reported from large lake enclosures (Turner & Rudd, 1983). Results of our microcosm studies are consistent with observations of increased bioaccumulation and toxicity of selenomethionine relative to selenate and selenite (Niimi & LaHam, 1976; Kleinow & Brook, 1986).

The persistence and bioavailability of dissolved Se species added to our microcosms have important implications for the fate and effects of Se in aquatic ecosystems. Differences in sediment affinity of dissolved Se species strongly affected their loss rates from water. However, selenite and selenomethionine were accumulated to higher concentrations in biota than selenate, despite being present at lower concentrations in water. These species may contribute disproportionately to Se bioaccumulation and toxicity in contaminated waters due to preferential uptake from water or transfer via food chain organisms. The very high BCFs of Se added as selenomethionine suggest the need for additional study of the occurrence and toxicological importance of organoselenium compounds. We are conducting additional studies of Se speciation in closed-system microcosms and Se bioaccumulation from organic and inorganic Se species in aquatic food chains.

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